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Using polyaspartic acid hydro-gel as water retaining agent and its effect on plants under drought stress

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Abstract Polyaspartic acid (PASP) hydrogel is an important and widely applied water-retaining agent, thanks to its special space network structure which contains a carboxyl group attached on the side chain. In this study, the PASP hydrogel with high water absorption rate (300–350 g H_2O/g hydrogel) was developed and adopted to transplant *Xanthoceras sorbifolia* seedlings in the ecological restoration project of *Mount Daqing* National Nature Reserve. Transplantation experiments showed that the survival rate and leaf water content index for *X. sorbifolia* seedlings were increased by 8–12% and 4–16%, respectively. Additionally, compared with the counterpart without PASP hydrogel, the value of chlorophyll fluorescence that was considered as one of the most important indicators of plant physiology, was significantly improved with the addition of PASP hydrogel. The PASP hydrogel displays a promising future for the applications of increasing the survival rate and simultaneously alleviating the drought stress effects on the pioneer plants in arid and semi-arid areas.

1. Introduction

Over the past decades, accompanied by climatic change and human activities, land degradation of the biological resources and destruction of the ecological balance become more serious every day, resulting in desertification and making the soil unsuitable for plant growth (Evans and Geerken, 2004). Especially, desertification is regarded as one of the most severe ecological environment problems in the world (Xu et al., 2009; Ashraf et al., 2012). Over 2/3 of the countries and regions have suffered the damage of desertification to a certain extent, and 1/4 of the land area is under the threat of desertification. Ecological restoration is another feasible approach to prevent and cure desertification compared with other methods like mechanical or chemical dune fixation (Wang et al., 2004) and biological soil crusts (Zheng et al., 2011; Xu et al., 2013; Ashraf et al., 2013). It can effectively reduce the desertification by growing plants that possess desert adaptation properties, which gradually improve the micro climate. Actually, the versatile characteristics of non-toxicity and high water adsorption rate of PASP hydrogel indeed lead to its wide applications as a water-retaining agent for the ecological restoration in the desert area. The plants grown in the desert area need to at least

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endure the drought. Previously, it has been reported that the addition of the water-retaining agents such as poly-acrylic acid salt, denatured starch (Bai et al., 2011; Hirotama et al., 2008) and cyanobacterial polysaccharides (Xu et al., 2013; Ashraf et al., 2015) could alleviate drought stress. However, apart from the water-retaining benefits, the reduced effect for the degradation products of the utilized chemicals on the environment and plant growth is also a critical issue (Prayogo et al., 2014). For instance, degradation of poly-acrylic acid in the natural environment is so difficult that a considerable environmental risk would appear with the large-scale application of poly-acrylic acid salt (Dumas et al., 1985; Serrano et al., 2015). For denatured starch, a large quantity of denatured starch is necessary to maintain the water retention effect due to its low water absorption rate. Therefore, the performance of a certain water-retaining agent should normally involve in the biodegradability, compatibility, and water adsorption as evaluation factors.

Polyaspartic acid (PASP), a macromolecular amino acid polymer taking aspartic acid as monomer, which is found naturally in many kinds of mollusks in vivo, possesses great biodegradability and biological compatibility. The PASP hydrogel was produced by crosslinking the hydrophobic PASP backbones. The PASP hydrogel has a much higher water adsorption capacity because of (I) the large number of carboxyl groups attaching on the PASP side chain that can bind water molecules, and (II) the space structure of PASP hydrogel that has the ability to absorb the free water in the environment (Qureshi et al., 2015).

In this study, we synthesized the PASP hydrogel using the purpose-designed pilot reactor. The fabricated PASP hydrogel was subsequently adopted as the water retaining agent on planting the pioneer plant – *Xanthoceras sorbifolia*, at the *nine peak* of *Mount Daqing* National Nature Reserve that has a Semi-arid climate.

It is worth mentioning that although there are extensive and in-depth studies on the level of laboratory research related to PASP hydrogel (Tan et al., 2002), the pilot reactor and large-scale applications of the PASP hydro-gel are still scarce so far. In this work, we designed the production on the basis of the synthetic route. The PASP hydrogel was synthesized by two steps: Firstly, polysuccinimide was hydrolyzed by the "solid phase synthesis" method. Secondly, the hydrolyzed polysuccinimide was cross-linked by glycol dimethyl glycidol ether (Lu et al., 2016). Similar reaction scheme is provided in Meng et al. (2015).

In order to evaluate the effect of the PASP hydrogel, the *nine peak* of *Mount Daqing* National Nature Reserve in China was selected as the experimental base. The average annual temperature is around 7.5 °C. The frost free period is about 117 days, and sunshine time is 3095 h. The annual precipitation and evaporation is 400 mm and 1300 mm, respectively. In addition, this selected area belongs to the semi-arid continental climate such that the plants in this area are largely under drought stress. The native species *X. sorbifolia* in the base was selected as the pioneer plant for environmental restoration, and the PASP hydrogel was chosen to promote the plant growth. Three methods were used for seedling transplantation: the field seeding seedlings which are less than one year old, the slope transplantation seedlings which are less than one year old and the slope transplantation for the annual seedlings. About 15,000 seedlings were transplanted in 30 hectares, which contains transplants with and without the PASP hydrogel (Tabassum et al., 2014).

Finally, we measured the growth index of seedlings by statistical methods. The physiological index of seedlings by chlorophyll fluorescence instrument was also determined. Effects of the PASP hydrogel used as a water-retaining agent in ecological restoration were finally illustrated.

2. Materials and methods

2.1. Materials

PASP hydrogel was produced by polysuccinimide, which has a water absorption rate about 300–400 g/g (the weight of water absorbed per gram of PASP hydrogel). Two kinds of *X. sorbifolia* seedlings were cultivated by Goldenrace Co.. The height of the seedlings *less than one year old* was around 15 cm, and the corresponding height for the *annual seedling* was about 150 cm. A FluorPen FP 100 hand-held chlorophyll fluorescence instrument, and Photon Systems Instruments, Czechia, were used to collect the data.

2.2. Toxicity determination of cross-linker on plant growth

The toxicity determination was based on China national standards (GB/T3543.4-1995, 1995), with toxicity of the rape seeds as the reference standard. The experimental plants and the rape seeds were firstly immersed in cross-linking agent for 24 h respectively, then cultivated at 25 °C for 7 days. The statistical germination rate was finally obtained (Safi et al., 2015).

2.3. Application of PASP hydrogel as water-retaining agent

For field seeding of *X. sorbifolia* seedlings less than one year old a smooth zone in the mountain valley was selected. A trench was plowed 15–20 cm deep and the PASP hydrogel was applied at 250 g/m. The seedlings were transplanted, backfilled, and compacted at 10-cm intervals.

The slope transplanting seedlings less than one year old are annual seedlings: a hole was dug at about 30 cm in diameter and depth, and the PASP hydrogel was applied at 25 g/hole. The *X. sorbifolia* seedlings were transplanted, and then backfilled and compacted with soil. The plant density is about 750–1200 trees/hectare.

2.4. Determination the growth index of X. sorbifolia seedlings

The survival rate is calculated as Formula (1)

$$M = \frac{M_s}{M_t} \quad (1)$$

where M_t and M_s are the total number and survival number of the cultivated seedlings, respectively.

Leaf relative water content (RWC) was estimated by the weight ratio of the current water content of the sampled leaves tissue relative to the maximal water content they can hold at full turgidity. Firstly, about 10–20 leaves were selected randomly. Dried leaf surface weight was regarded as M_1.

Then these leaves were immersed into distilled water for 8 h in order to reach the water saturated state. The water saturated leaves were taken out and the excess water on the surface was removed, and the present weight was determined as M_2. Subsequently, these leaves were heated at 105 °C for 20 min to inactivate the enzyme in plants. Finally, the annealed leaves were removed into an oven at 80 °C to dry the leaves until a constant weight M_3 was obtained. The leaf RWC was expressed as Formula (2)

$$\mathrm{RWC} = \frac{(W_1 - W_3)}{(W_2 - W_3)} \tag{2}$$

2.5. Determination the chlorophyll fluorescence value of *X. sorbifolia* seedlings

After transplanting the *X. sorbifolia* seedlings for 3 months, the physiological indexes become relatively stable and the physiological activity reached the maximum. The chlorophyll fluorescence value of seedlings was then measured by chlorophyll fluorescence instrument. Specially, 1–2 full expanded leaves from different strains were selected randomly for detection. The maximum photochemical quantum yield (QY) of the optical system II (PSII) is defined by fluorescence. The minimal fluorescence (F_0) was obtained by putting the leaves into a light and dark adaptation for 20 min, then using the weak light for illuminating. After that, the leaves were illuminated by a saturation pulse light (4000 μmol m^{-2} s^{-1}), which was closed after a pulse, to get the maximum fluorescence (F_m). The QY of PSII was calculated according to Formula (3).

$$\mathrm{QY} = \frac{(F_m - F_0)}{F_m} \tag{3}$$

The fast chlorophyll fluorescence induction kinetics curve (OJIP) and the initial slope M_0 were determined by the fluorescence values at different time points. Concurrently, the active parameters of the unit PSII reaction center were obtained by system automatic calculations in the selected mode. In this paper, the absorbed light energy (ABS/RC), the captured energy for reduction of QA (TR_0/RC), the captured energy for electron transfer (ET_0/RC), and the consumed energy (DI_0/RC) were all included as active parameters.

3. Results and discussions

3.1. Toxicity determination of cross-linker on plant growth

As an amino acid polymer, PASP with great biocompatibility has been widely used as the accelerator and fertilizer synergist in plants. However, the effect for the toxicity of the cross-linker on plant growth should also be considered since the residual cross-linker may cause environmental damage in the ecological restoration process. Here, the statistical germination rate of the rape seeds with cross-linker was 94%, which was slightly lower than the one without the cross-linker (98%). Therefore, the results revealed that the cross-linker did not significantly harm plant growth, which could be utilized for PASP hydrogel production.

3.2. Effect of PASP hydrogel on physiological index of *X. sorbifolia* seedlings

The superior effect for the addition of PASP hydrogel on the survival rate and the leaf relative water content of *X. sorbifolia* seedlings was demonstrated in Table1. In details, the survival rate of the seedlings transplanted with the help of PASP hydrogel was increased by 8–12% for different transplantations. Besides, it was obvious that the leaf relative water content for the seedlings transplanted in mountainous areas with the PASP hydrogel was also improved than the ones without PASP hydrogel, the former has a value 150% higher than the latter. Thus, by adding the PASP hydrogel, it is undoubtedly true that both the plant survival rate and leaves relative water content were significantly improved, especially the ones planted in the drought stress region.

3.3. Effects of PASP hydrogel on chlorophyll fluorescence value of *X. sorbifolia* seedlings

Photosynthesis was the most important process in plant growth, during which light energy mainly converts into chemical energy, while a minor part is released as a longer fluorescence. It is well known that the chlorophyll fluorescence signals emitted by the plant reflect much information on photosynthesis. As photosynthesis has a close relationship with nutrition and stress degree of the plants, the detection and analysis of the chlorophyll fluorescence signal contributes greatly to a better understanding of the growth, disease, stress, and the physiological status of the plants (Baker, 2008). Therefore, in the present paper, the chlorophyll fluorescence value was taken as the physiological index of *X. sorbifolia*, and the effect of the PASP hydrogel on the seedling growth under drought stress was investigated systematically.

3.4. Maximum photochemical quantum yield

The QY value was tested at 10:00 a.m., 1:00 p.m., and 4:00 p.m., respectively. The leaves were put into the light and dark adaptation. As can be seen in Figs. 1 and 2, the QY value under the non-stress condition was about 0.8–0.85 (Björkman and Demmig, 1987; Johnson et al., 1993), which

Table 1 Survival rate and leaf relative water content for *Xanthoceras sorbifolia* seedlings.

		Survival rate (%)	Leaf relative water content (%)
The field seedling for less than one year old seedlings	PASP	81.65	28.26
	blank	73.38	20.43
The slope transplantation for less than one year old seedlings	PASP	61.73	27.86
	blank	51.55	11.71
The slope transplantation for annual seedlings	PASP	69.32	17.48
	blank	57.80	14.85

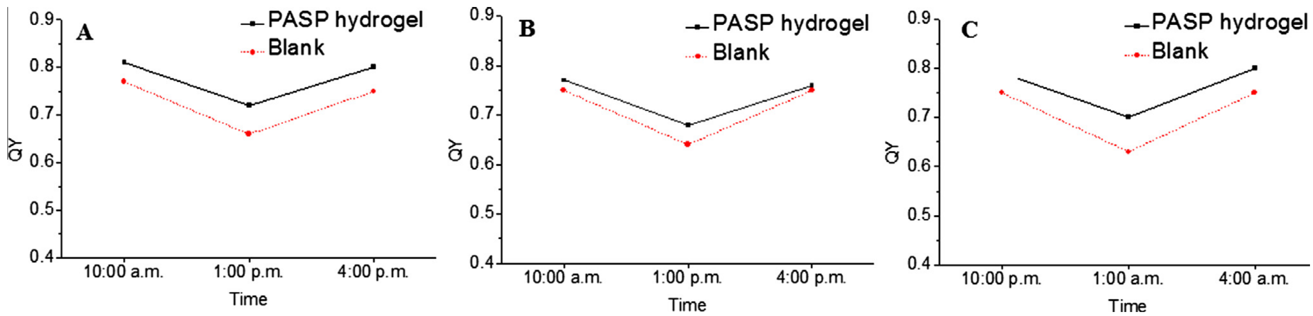


Figure 1 Photon yields of the *Xanthoceras sorbifolia* seedlings under dark adaptation. (A) The field seeding of seedlings less than one year old. (B) The slope transplantation of seedlings less than one year old. (C) The slope transplantation of the annual seedlings.

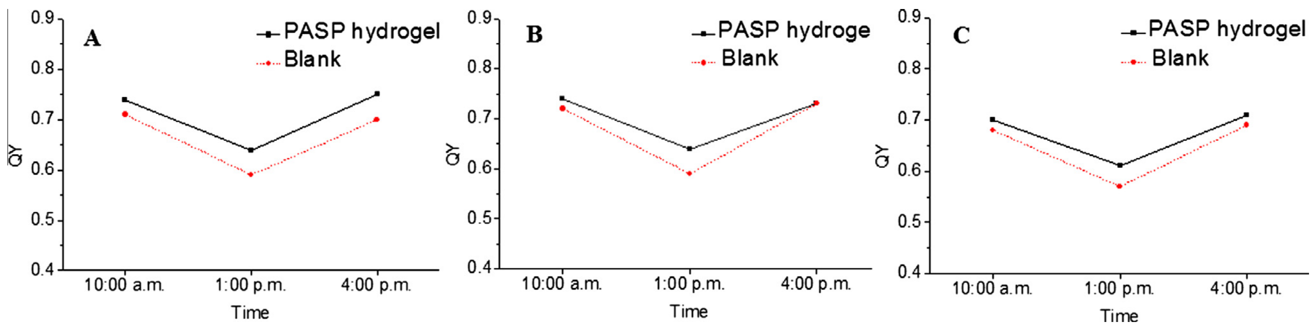


Figure 2 Photon yields of the *Xanthoceras sorbifolia* seedlings under light adaptation. (A) The field seeding of seedlings less than one year old. (B) The slope transplantation of seedlings less than one year old. (C) The slope transplantation of the annual seedlings.

reflects no effect on the growth condition occurs in this case. On the contrary, the QY value under the stress condition decreased significantly, indicating the reduced conversion efficiency from the solar energy. It is also shown here that the QY value of *X. sorbifolia* transplanted with the addition of PASP hydrogel was much higher than the ones without the PASP hydrogel regardless of the transplanted conditions. In short, the results illustrated that the addition of the PASP hydrogel could slow down the decline of photosynthesis efficiency caused by drought stress of the plants, resulting in the relief of drought-stress on plant growth.

The QY values of transplanting *X. sorbifolia* seedlings with PASP hydrogel decreased slightly and slowly around 1:00 p.m. in the strong sunshine. The QY value reflects the variation of chlorophyll PSII activity under different light intensities. In conditions under strong light, the PSII activity decreased and the photosynthesis photoinhibition effect was enhanced, thus the QY value decreased consequently. The present results illustrated that the addition of the PASP hydrogel can reduce, to some extent, the photosynthesis efficiency loss in the strong sunlight.

3.5. Fast Chlorophyll fluorescence induction kinetics curve

The fast chlorophyll fluorescence induction kinetics curve was studied after full dark adaptation (Maxwell and Johnson, 2000). As can be seen in Fig. 3, the fluorescence value using different transplanting methods was improved with the addition

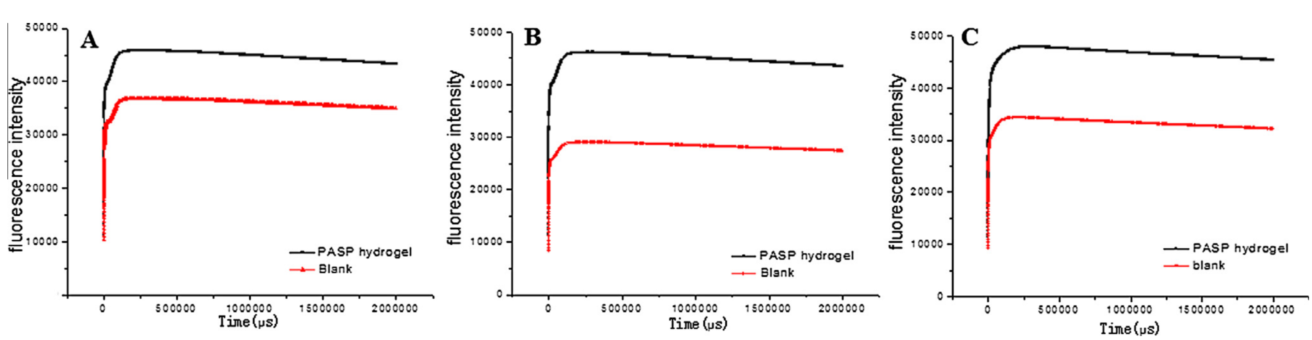
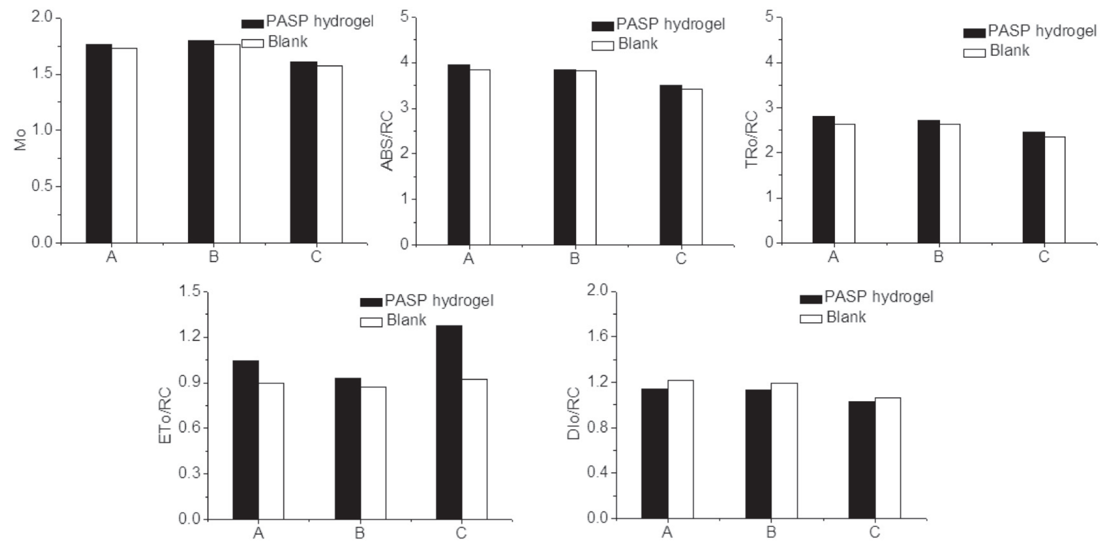


Figure 3 Fast Chlorophyll fluorescence induction kinetics curve of the *Xanthoceras sorbifolia* seedlings under light adaption. (A) The field seeding of seedlings less than one year old. (B) The slope transplantation of seedlings less than one year old. (C) The slope transplantation of the annual seedlings.



A: The field seeding of seedlings less than one year old.

B: The slope transplantation of seedlings less than one year old.

C: The slope transplantation of the annual seedlings.

Figure 4 The activity parameters of PSII reaction center of the *Xanthoceras sorbifolia* seedlings under light adaptation.

of the PASP hydrogel than the one without PASP hydrogel, which is in agreement with our previous results. The fast chlorophyll fluorescence induction kinetics curve showed that the addition of PASP hydrogel can effectively slow down the declining plant photosynthesis rates, especially the ones planted in the drought stress region.

3.6. The activity of PSII reaction center

The slope M_0 and the activity of PSII reaction center (various quantum efficiency of the active unit reaction center (RC)) (ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC) can be calculated from the fast chlorophyll fluorescence induction kinetics curve (Strasser, 1995; Bussotti et al., 2007). The analysis of M_0, ABS/RC, TR_0/RC, ET_0/RC, and DI_0/RC promote a better understanding of the absorption, transformation and consumption status of the light energy. M_0 value that is related to the reaction center pigments and QA state reflecting the maximum rate of reduction of plastoquinone QA, are demonstrated in Fig. 4. M_0 value of the transplanted seedling with the PASP hydrogel was higher than the one without PASP hydrogel, which means the addition of PASP hydrogel could increase the activity of QA and decrease the effect of drought stress on *X. sorbifolia* seedlings. Meanwhile, the addition of PASP hydrogel also improves the ABS/RC, TRO/RC, ETO/RC values of the unit reaction center. The partial inactivation or cleavage of the reaction center on the blade unit area occurred under drought stress. It illustrated that the addition of PASP hydrogel could slow down the deactivation degree of the active centers and decreases the effects of drought stress on *X. sorbifolia* seedlings. However, the addition of PASP hydrogel will reduce the DIO/RC value, demonstrating that the addition of PASP hydrogel could also decrease the energy consumption of the active reaction center, i.e. the efficiency will be increased.

4. Conclusions

A designed pilot production of PASP hydrogel with a high water absorption rate of 300–350 g/g PASP hydrogel was utilized in this study. The assessment of a cross-linker presented on seed germination rate demonstrated that the cross-linker has no effect on the plant growth. In the ecological restoration project of *Mount Daqing* National Nature Reserve, the *X. sorbifolia* seedling was transplanted with and without water-retaining PASP hydrogel experimentally. With the addition of the PASP hydrogel, the survival rate and the leaf relative water content were found to increase by 8–12% and 4–16%, respectively. The physiology index which was calibrated by chlorophyll fluorescence value was also significantly improved. All results illustrated that the PASP hydrogel can be used in ecological restoration as well as soil and water conservation projects in arid and semi-arid areas thanks to its high water absorption, water retention property and biocompatibility. The applications of PASP hydrogel could alleviate the drought stress effect on the pioneer plants, promote the growth of pioneer plants and increase the survival rate, which is believed to have a promising application prospect.

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